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Supplemental Material to:

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CpG dinucleotide-specific hypermethylation of the TNS3 gene promoter in human renal cell carcinoma

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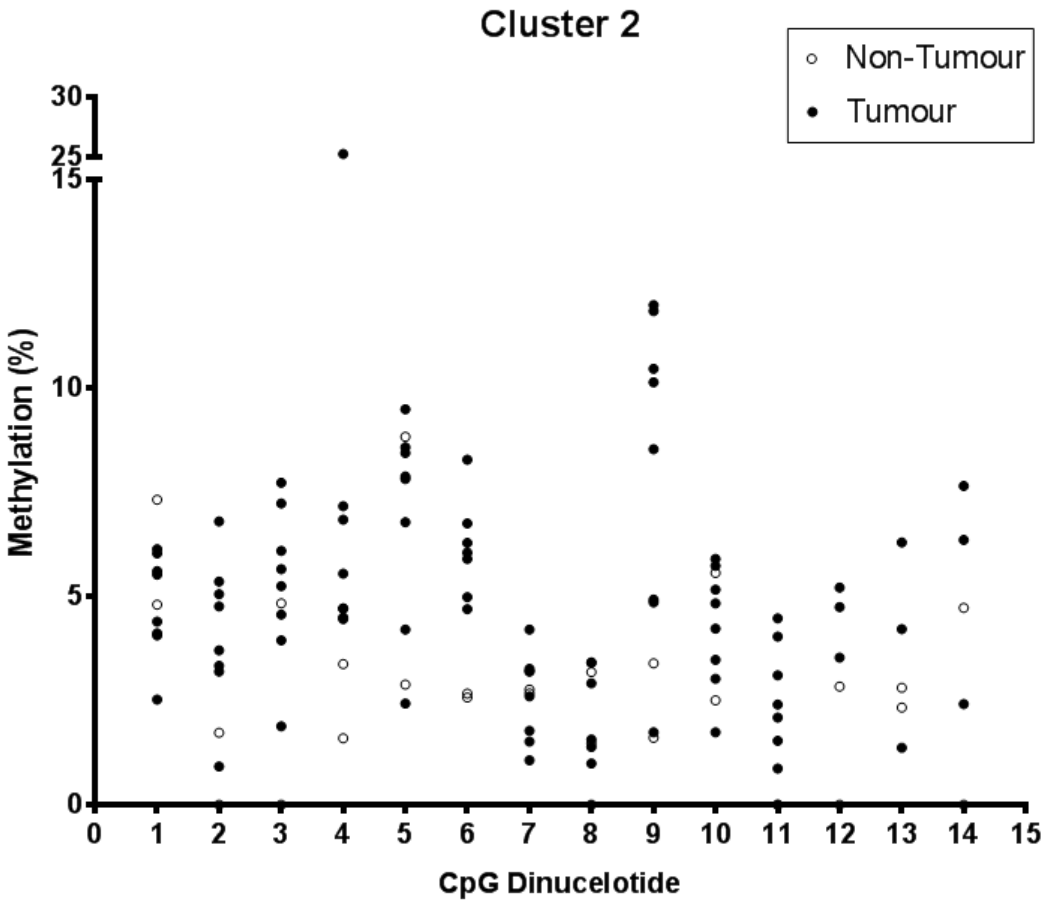
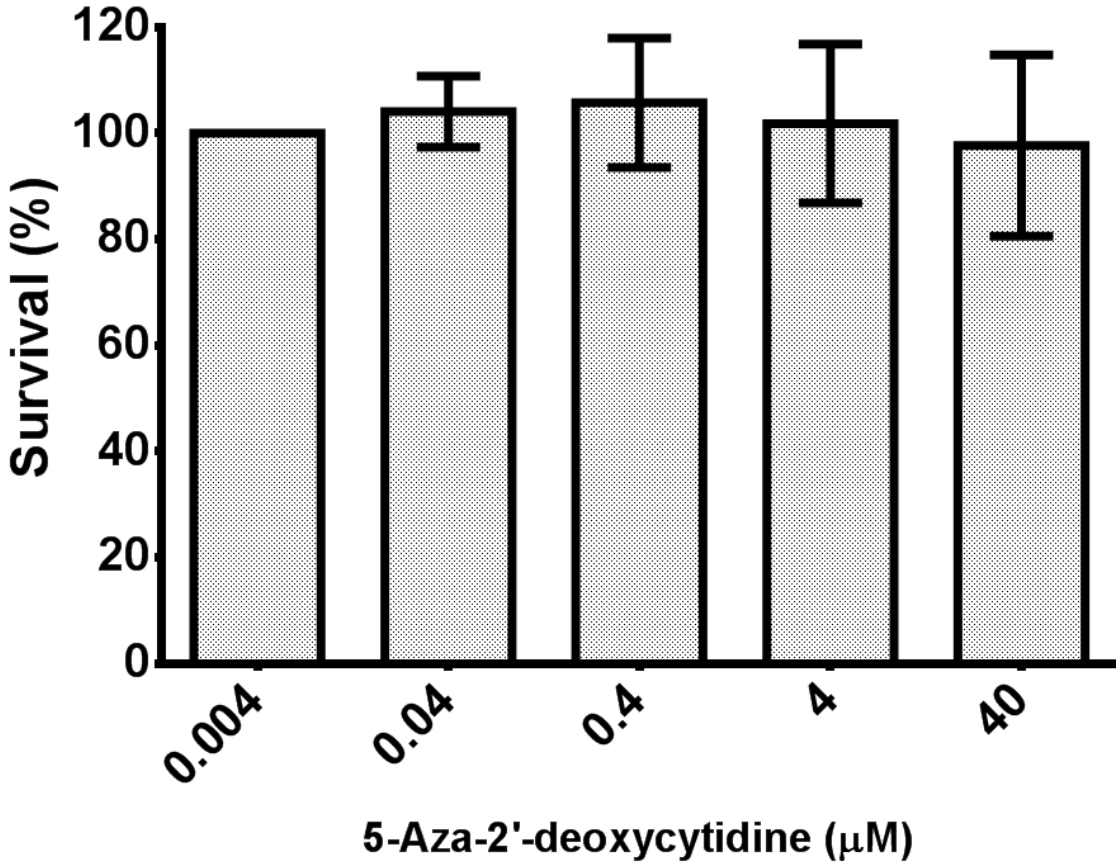
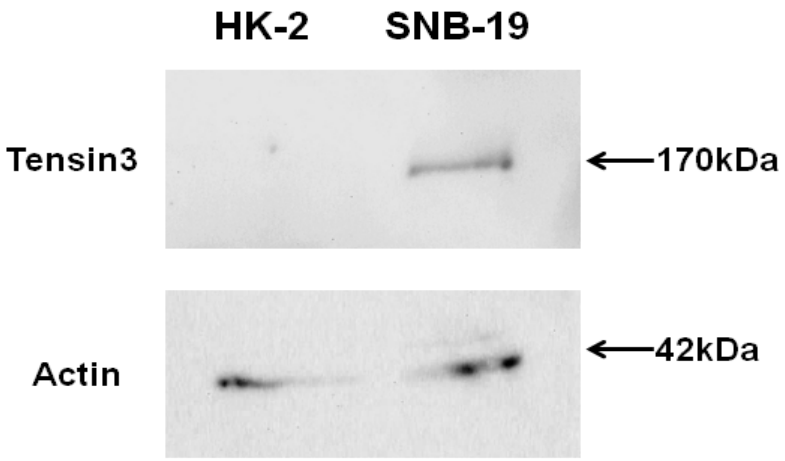


Supplemental Figure 1. The *TNS3* CpG island cluster 2 region was sequenced using one PCR product, using a sequencing primer that covered 14 CpG dinucleotides. Dots represent the methylation level (%) of an individual patient sample at each CpG dinucleotide, with black dots denoting RCC tumour samples (n=8) and white dots denoting adjacent non-tumour kidney samples (n=2).

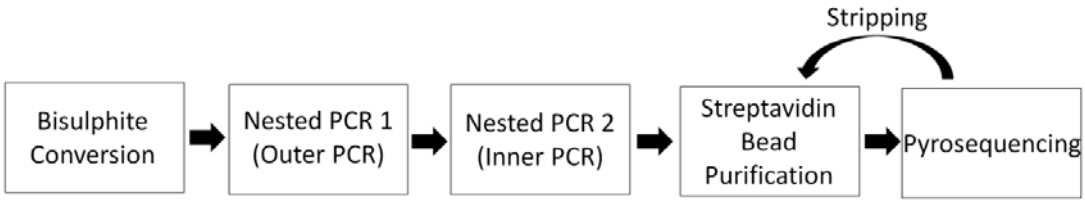


Supplemental Figure 2. Effect of 5-aza-2'-deoxycytidine on cell viability. Human 786-O kidney cancer cells in serum free medium were incubated with different concentrations of 5-aza-2'-deoxycytidine at for 72h. Conversion of the MTS formazan dye was used as an index of cell viability. No significant difference in viability was observed at any of the treatments; bars represent mean±SEM % cell viability (n=3).

Supplemental Figure 3. The effect of 5-aza-2'-deoxycytidine treatment on Tensin2 mRNA expression in human kidney cells. HEK293 cells were treated with 40μM 5-aza-2'-deoxycytidine (5-aza) for 96h, and RNA extracted from cell lysates underwent qRT-PCR analysis of Tensin2 mRNA expression. Bars represent mean±SD of gene expression relative to untreated control;** denotes $P<0.01$ (n=2, representative of 3 experiments).



Supplemental Figure 4. Western blot detection of Tensin3 protein. Human kidney HK-2 (lane 1) and human glioblastoma SNB-19 (lane 2) cell lines were run on SDS-PAGE. After transfer, the membrane was probed with a specific in-house anti-human Tensin3 antibody to detect the Tensin3 protein, revealing a band at approximately 170kDa (upper image). Also, the 42-kDa bands for actin in the same samples are shown in the lower portion of the same blot (lower image).



Supplemental Figure 5. Pyrosequencing work flow. Genomic DNA first underwent bisulphite conversion, where the converted DNA acts as template for a nested PCR reaction sequence. The biotinylated terminal of the PCR product enables it to be purified against streptavidin beads, and rendered single stranded so as to act as template for pyrosequencing using a sequencing primer. Following pyrosequencing, the same sample can be used to analyse another region, in which case the sample undergoes the affinity purification procedure once more and is again prepared as a single-stranded template for pyrosequencing, this time using another sequencing primer.

Supplemental Table 1. Primers used in the pyrosequencing workflow.

Primer	Forward primer (5'-3')	Reverse primer (5'-3')
Nested PCR primer (outer)	GGGTTGTTTAATGGGTTTGATAAT	ACCTACCCATTAAAACCCCAACT
Nested PCR primer (inner) - cluster 1		CCCAATACCTAAAAAAACCTAAAAC (biotinylated)
Nested PCR primer (inner) - cluster 2	TGGGGAGAGTATAGAGATTTGGAG	CCCAAACCCAACCAATCA (biotinylated)
Sequencing primer 1 (cluster 1)	GATGAGGTTAGGTGATT	
Sequencing primer 2 (cluster 1)	GTAGTTAGTTAGGA	
Sequencing primer 3 (cluster 2)	GGAGGAATTTAGGTTAGGGA	

Supplemental Table 2. Primers for luciferase assay constructs.

PCR product as portion of *TNS3* promoter	Forward primer (5'-3')	Reverse primer (5'-3')
500bp	GACTGGTACCGGGGAGCGCGGAGACAGA	CTCGAGTGGTCCTGCGCCCCAGCCAGTCC
1000bp	GCAGCCTCCAGACCACTTGGAGCCTGGG	CTCGAGTGGTCCTGCGCCCCAGCCAGTCC
2000bp	ACTCGCTTCCTGGTGTCTCCTAGAGTGTA	CTCGAGTGGTCCTGCGCCCCAGCCAGTCC